REMARKS

In the Office Action dated January 24. 2003 and the Office Action dated July 1, 2003, claims 14 and 60-77, in the above-identified U.S. patent application were rejected. Reconsideration of the rejections is respectfully requested in view of the above amendments and the following remarks. Claims 14 and 60-77 remain in this application and claims 1-13 and 15-59 have been canceled.

The diagrams attached to the April 16, 2003 response show the differences between the claimed invention and the cited prior art. As indicated on the diagrams, in the present invention PEG protects the antibody from unspecific interactions and therefore no undesired side reactions or by-products are produced. In contrast to this, the diagrams indicate that in Herron, PEG acts as a spacer for binding to the solid surface and does not provide protection from unspecific interactions. There does not appear to be any inconsistency between these diagrams and the arguments which were previously made (i.e. that Herron covers the entire surface with glutaraldehyde and then PEG resulting in a PEG spacer). The third embodiment of Herron pointed out in the office action is also based on a glutaraldehyde modified surface (i.e. a surface containing a CHO group) which is reacted with a PEG spacer which as shown in the diagrams is different from the present invention where PEG protects the antibody from nonspecific interactions and thus activation of the surface with glutaraldehyde is not necessary. Activation with glutaraldehyde is necessary in Herron because the PEG is acting as a linker.

In addition, as indicated in the prior response, claim 14 indicates that the conjugate is formed prior to immobilization (hence the term "preformed") and thus is different from a method where the individual components of the conjugate are sequentially immobilized as suggested in the office action. The preformation of a PEG antibody conjugate is not possible with Herron's method since a bifunctional spacer is used which can react with both functions with an oxidized polyfunctional antibody molecule containing aldehyde groups. Thus, contrary to statements made in the office action, the individual members of the conjugate are not sequentially immobilized on the solid phase.

The present invention is a method for the detection of an analyte in a sample. The assay is a heterologous assay wherein the binding of the analyte to a solid phase via an analyte specific reactant is determined. According to the present invention, the analyte specific reactant is provided as a conjugate with a poly (C_2-C_3)-alkylene oxide which is subsequently immobilized on the solid phase. The use of this preformed conjugate results in several advantages as shown in the examples in the present application and discussed below.

Examples 9 and 10 disclose the preparation of analyte-specific reactant (antibody)-polyethylene glycol conjugates and their subsequent immobilization on the solid phase.

Example 11 demonstrates several advantages of the present invention. In 11.1 it is shown that the use of antibody-PEG conjugates leads to a decrease in the blank value on solid phases. 11.2 shows that the unspecific binding of buffer components is

substantially decreased. 11.3 shows that the unspecific binding of human antibodies is decreased.

Examples 12 and 13 demonstrate that the determination of anti-HIV antibodies in a sample may be improved when a PEG conjugate is used as a solid phase antigen.

Example 14 demonstrates that an antibody-polyethylene glycol conjugate leads to an improved detection of HBS antigen.

The allegedly closest prior art documents are Herron '492 and '196. These references describe a solid phase assay, wherein a bifunctional PEG molecule (PEG-$(ED)_2$) is coupled to the solid phase. The bifunctional PEG molecule has a reactive amino group on each end. One of these amino groups shall react with an aldehyde group on the solid phase, the other one shall remain in reactive form in order to subsequently react with an oxidized antibody. The manufacture of this surface is described in detail in Example III of Herron '196 or '492. As can be gathered from Table I (Herron '492, col. 13 and 14), the resulting surface has a comparatively low binding, a high non-specific binding and a low specific activity compared to other methods of immobilization, e.g. an avidin/biotin immobilization. Consequently, the method described in Example III does not produce good results (Herron '492, col. 9, lines 42-44). In particular, the method of Example III gives a higher level of non-specific binding ('492, col. 9, lines 49-50).

Section I of the previously submitted diagrams, describes the method of the present invention. The PEG antibody conjugate is preformed by reacting a monofunctionalized PEG derivative with a biotinylated antibody having reactive amino

groups. The number of PEG groups on the antibody can be adjusted over a broad range of using respective stoichiometric rations of PEG to antibody. The resulting products is an immobilized antibody which is surrounded by PEG groups, and thus, protected from unspecific interactions with sample or buffer components.

Section II illustrates the method according to Herron. Herron starts with a bifunctional PEG derivative which is reacted with a glutaraldehyde-activated solid phase carrying CHO groups. Due to its bifunctional nature a large proportion of the bifunctional PEG molecules will react with the surface in a way that both amino groups form bonds with aldehyde groups. Thus, a PEG bridge is obtained which is an undesired by-product, since the binding of an antibody molecule thereto is no longer possible. Only a fraction of the bifunctional PEG molecules react in a way that one free amino group remains which is capable of subsequent reaction with an antibody. In the resulting product the antibody is bound to the surface via a PEG spacer. The PEG group is not available for surrounding the antibody molecule and, thus, does not provide protection from unspecific interactions with sample or buffer components.

Section III of the diagram clearly shows that the prior art method of Herron cannot be used in a method where a PEG antibody conjugate is preformed and subsequently immobilized on the surface.

When reacting the bifunctional PEG derivative with the oxidized antibody a great number of undesired by-products would result due to the polyfunctional nature of the reaction partners. For example, the bifunctional PEG molecule might react with two different antibody molecules, resulting in a crosslinked antibody (I) which would no

longer be capable of reacting with a glutaraldehyde-modified surface. Alternatively, both amino groups of the bifunctional PEG molecules would react with aldehyde groups on the oxidized antibody, resulting in an intramolecular reaction product (ii), which could not react with a glutaraldehyde-modified surface either. A person skilled in the art could not modify the method as described in the Herron references by preforming a PEG conjugate of an analyte-specific reactant which would subsequently be immobilized on a solid phase.

The office action indicates that claim 14 encompasses immobilization by any method including a PEG spacer. Applicants respectfully point out that immobilization with a preformed PEG antibody conjugate is not possible according to Herron's method as shown in the previously submitted diagrams and discussed above. The reaction of a PEG-ethylene diamine and an oxidized Fab' molecule would yield a polymeric crosslinked Fab'-PEG conjugate which can no longer be immobilized.

The office action points out a clerical error regarding the term "binding" in claims 64 and 65. Claims 64 and 65 were previously amended correcting this error.

The office action indicates that claims 64 and 65 are indefinite because they recite "the solid phase is immobilized" while claim 14 recites that the "conjugate" is immobilized. Claims 64 and 65 were amended in the April 16, 2003 response to recite "conjugate".

The office action indicates that there is no antecedent basis in claim 14 for the language "conjugate of the modified solid phase reactant with the second partner of the binding pair" and "the modified analyte specific solid phase reactant". This language

was deleted from claims 66 and 68 in the prior response. Claim 66 and 69 currently refer to the "preformed conjugate".

The office action indicates that the language "non-analyte specific molecules" in claim 69 is unclear. Claim 69 was amended in the prior response to delete this language.

The office action indicates that the language "a further alkylene oxide modified binding molecule" in claim 68 is unclear. In the prior response, claim 68 was amended to recite ""a further alkylene oxide modified solid phase reactant" in order to clarify what the "binding molecule" is.

The office action states that the record does not indicate that the term "test reagent" was changed to "detection reagent". Applicants point out the amendment filed on September 3, 2002 where claim 14 was amended to recite "detection reagent". Claims 60-62 and 64-77 directly or indirectly refer back to claim 14 and claim 63 never recited the term "test reagent". The request for continued examination filed on October 31, 2002 requested that the amendments filed on September 3, 2002 be entered. Support for the language "detection reagents" can be found at page 11, lines 8-18 of the application regarding a modified soluble analyte specific reactant which is labeled and examples 13 and 14 in the application which use labeled reagents to detect the analyte. Applicants also point out that the term "test reagent" is disclosed on page 1, second paragraph, line 4.

The office action indicates that the term "said preformed conjugate" has no antecedent basis in claim 14. This is incorrect as claim 14, part (a) recites "preparing a

solid phase on which a preformed conjugate of a poly (C_2-C_3)-alkylene oxide ..." (See the September 3, 2002 amendment).

The office action states that the meaning of the term "an alkylene oxide modified blocker" has not been established. Page 4 (first paragraph) of the April 16, 2003 response indicates that pages 4 and 5 of the application discuss an alkylene blocker, suitable blocking agents, and binding of the blocker to the solid phase. In addition, page 4, line 27 to page 6, line 29, discuss blockers and preferred alkylene oxide blockers are described at the bottom of page 5 to page 6. In view of this disclosure, applicants contend that the term "an alkylene oxide modified blocker" has been clearly defined.

The office action states that claim 77 is unclear. The prior office action stated that is was unclear whether the "different analyte-specific solid phase reactants" were the same as the "conjugates" of claim 14. In the April 16, 2003 response, applicants amended claim 77 to indicate that the preformed conjugates (as recited in claim 14) are immobilized in several test areas. In order to further clarify this issue, claim 14 part (a) has been amended to indicate that the preformed conjugate is immobilized in a test area. This amendment is supported by the disclosure on page 3 of the present application which discusses defined test areas. Applicants point out that the term "test area" does not necessarily mean a limited section of the carrier.

The office action indicates that the language "an analyte-specific modified solid phase reactant" in claim 60 is unclear. In the prior response claim 60 was amended to

delete this language and recite "said analyte specific reactant is conjugated with a member of a high affinity binding pair" in order to clarify how the reactant is used.

Claim 61 was rejected as incorrectly characterizing the "modified solid phase reactant" as being "antibodies, antigens, nucleic acids....". In the prior response claim 61 was amended to indicate that the "analyte specific reactant is selected from analyte specific antibodies, antigens, nucleic acids, ...".

The office action indicates that support has not been provided for the amendment to claim 60 and that it is unclear whether the "member of the high affinity binding pair" is attached directly to the solid phase. Applicants point out page 4 of the present application which describes the analyte-specific solid phase reactant in the first paragraph and discusses immobilization by conjugating the solid phase reactant with a member of a high affinity binding pair in the second paragraph. In order to clarify this issue, claim 60 has been amended to indicate that the solid phase is coated with a first member of a high affinity binding pair and the analyte specific reactant is conjugated with a second member of said high affinity binding pair, wherein said preformed conjugate is immobilized via said high affinity binding pair.

Claim 63 was rejected under 35 USC §112, first paragraph, as containing new matter regarding the term "preformed conjugate". Applicants point out examples 9 and 10 which disclose the preparation (preformation) of the conjugates and their subsequent immobilization on the solid phase. Regarding support for claims 60-62 and 64-77, applicants point out page 4, lines 10-26, which discusses immobilization by direct absorptive binding, covalent coupling or coupling via high affinity binding pairs, lines 27-

32, which discusses an alkylene blocker, page 5, lines 3-8, which discloses suitable blocking agents and binding of the blocker to the solid phase, page 3, lines 27-31, discusses suitable solid phases, and the examples discuss analyte specific regions immobilized on spatially limited test areas.

Claims 14 and 64 were rejected under 35 USC §102(b) as anticipated by Herron '492 or Herron '196. Claims 60-77 were rejected under 35 USC §103 as obvious over Herron '492 or Herron '196. As discussed above, Herron attaches the antibody to the solid surface using a PEG spacer but does not conjugate multiple PEG groups to the antibody to prevent non-specific interactions. Claim 14 currently requires a preformed conjugate to be applied to the solid phase which excludes Herron's process as a preformed PEG spacer-antibody conjugate is not possible due to intramolecular reactions. The disclosure cited in the office action (col. 16, lines 38-45 of Herron '196) clearly indicates that the PEG molecules were coupled to the silica surface (lines 38-40) and the other ethylenediamine group is later coupled to the antibody (lines 43-45). Herron '492 (col. 4, lines 2-5) indicates that the surface is coated with the derivatized PEG and later reacted with the Fab' capture molecules. Binding the PEG to the solid surface first, produces a different product than binding the PEG to the antibody first. In view of these differences, applicants request that this rejection be withdrawn.

Claims 14 and 64 were rejected under 35 USC §102(b) as anticipated by Caldwell '703 or Caldwell '503. Claims 60-77 were rejected under 35 USC §103(a) as unpatentable over Caldwell '703 or Caldwell '503. Caldwell '703 discloses a method where a derivatized Pluronic surfactant (a PPO/PEO copolymer) is adsorbed onto a

hydrophobic surface. The entire hydrophobic surface is coated first and later, a biomolecule is immobilized on the pretreated solid surface via the surfactant (col. 9, lines 27-28). Thus, the polymeric surfactant is not conjugated to the antibody prior to immobilization on the solid surface, the polymeric surfactant is immobilized first in a process similar to Herron's. Caldwell '503 describes the coating of hydrophobic surfaces with a molecule referred to as end-group activated polymer (EGAP). Similar to Herron '492 the entire surface is coated with said EGAP. Subsequently, a biomolecule is covalently bound to the precoated surface. This procedure is discussed in several places in '503, col. 4, lines 50-55, column 6, lines 19-24 or example 2, col. 19, lines 7-25. Caldwell'503 clearly indicates in claim 1 that the copolymer is adsorbed to the hydrophobic surface prior to conjugation with a biomolecule. Thus, Caldwell '503 and '703 do not disclose a "preformed conjugate". In Caldwell '503 and '703 the solid phase is treated to suppress unspecific binding to the solid phase. In contrast to this, in the present invention the reactant is treated and a conjugate is prepared which is subsequently coated as shown in the previously submitted diagrams. In view of these differences, applicants request that this rejection be withdrawn.

Applicants respectfully submit that all of claims14 and 60-77 are now in condition for allowance. If it is believed that the application is not in condition for allowance, it is respectfully requested that the undersigned attorney be contacted at the telephone number below.

In the event this paper is not considered to be timely filed, the Applicant respectfully petitions for an appropriate extension of time. Any fee for such an

extension together with any additional fees that may be due with respect to this paper, may be charged to Counsel's Deposit Account No. 02-2135.

Respectfully submitted,

By _____

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